Trabajo

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Growing towards disparity: geometric morphometrics reveals sexual and allometric differences in *Aparasphenodon brunoi* (Anura: Hylidae: Lophyohylinae) head shape

Roberta Azeredo Murta-Fonseca[1,2], Manuella Folly[2], Luiz Fernando Carmo[2], Angele Martins[2,3]

[1] Laboratório de Zoologia, Campus do Pantanal, Universidade Federal do Mato Grosso do Sul, Avenida Rio Branco 1279, Bairro Universitário, Corumbá, MS, CEP 79304-902. Brazil.

[2] Departamento de Vertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, Rio de Janeiro, RJ, CEP 20940-040. Brazil.

[3] Departamento de Ciências Fisiológicas, Instituto de Ciências Biológicas, Universidade de Brasília, Campus Darcy Ribeiro, Asa Norte, Brasília, DF, CEP 70910-900. Brazil.

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ABSTRACT

Sexual dimorphism and allometry feature is the main categories of intraspecific variation found in the animal kingdom, and techniques such as Geometric Morphometrics are effective to investigate variations in shape. Considering that many vertebrates exhibit substantial allometry and sexual dimorphism, we test the hypotheses that (1) *Aparasphenodon brunoi* displays ontogenetic and static allometry; and that (2) there is sexual dimorphism in head shape in this species. We analyzed 75 specimens of *A. brunoi* from Parque Nacional da Restinga de Jurubatiba and found significant ontogenetic and static allometries in head shape, as well as sexual dimorphism after size correction. Regarding sexual dimorphism, females tend to have larger heads, besides slightly anteriorly positioned nostrils, smaller eyes, larger canthal ridges, narrower and longer frontoparietal ridges, and larger and laterally shifted squamosal ridges. We discuss the relevance of head morphology variation in the species, as well as the presumed consequences on the species fitness.

Key words: Casque-headed frog; Sexual dimorphism; Allometric variation; Intraspecific variation; Ontogeny.

RESUMO

Dimorfismo sexual e alometria são as principais categorias de variação intraespecífica encontradas em metazoários e técnicas como a Morfometria Geométrica são eficazes para investigar variações na forma. Considerando que muitos vertebrados exibem considerável alometria e dimorfismo sexual, testamos as seguintes hipóteses: (1) *Aparasphenodon brunoi* exibe alometria ontogenética e estática; e (2) existe dimorfismo sexual na forma da cabeça nesta espécie. Analisamos 75 espécimes de *A. brunoi* do Parque Nacional da Restinga de Jurubatiba e encontramos alometrias ontogenética e estática significativas na forma da cabeça, além de dimorfismo sexual após a correção do tamanho. Em relação ao dimorfismo sexual, as fêmeas tendem a ter cabeças maiores, além de narinas ligeiramente posicionadas mais anteriormente, olhos menores, cristas cantais maiores, cristas frontoparietais mais estreitas e mais longas e cristas esquamosais maiores e deslocadas lateralmente. Discutimos a relevância da variação da morfologia da cabeça na espécie, bem como as consequências presumidas na aptidão da mesma.

Palavras-chave: Perereca-de-capacete; Dimorfismo sexual; Variação alométrica; Variação intraespecífica; Ontogenia.

Introduction

Intraspecific differences in size, shape or other external traits between females and males of a given species are traditionally defined as secondary sexual dimorphism (Shine, 1994; Freeman and Heron, 2007), and such differences may be driven by sexual selection (Darwin, 1871), ecological selection (Shine, 1979) or both. Allometry – the dependence of shape on size (Klingenberg, 2010, 2016) – and sexual dimorphism feature as the main categories of intraspecific variation found in the animal kingdom

Autor para correspondencia: roberta.m.fonseca@ufms.br

(Bolnick and Doebeli, 2003), and might be associated with an increase in ecological opportunities for individuals (Schluter, 2000; Agrawal, 2001; Lorch *et al.*, 2003; Lisle and Rowe, 2015). These evolutionary features can be applied to predict species diversification, since sexual selection can promote species reproductive isolation. Such isolation consequently cements speciation and increase the rate of adaptation to a novel environment, with a powerful synergy between natural and sexual selection that can elevate population mean fitness (Schluter, 2000; Agrawal, 2001; Lorch *et al.*, 2003; Lisle and Rowe, 2015).

Amphibians are often sexually dimorphic in several features such as body shape, color, morphology, ornaments, physiology (Kupfer, 2007; Bell and Zamudio, 2012), and, more conspicuously, in body size (see Monnet and Cherry, 2002; Kupfer, 2007). Although several recent studies have assessed amphibians' allometry through the use of geometric morphometrics (e.g., Fratani *et al.*, 2018; Duport-Bru *et al.*, 2019), studies regarding sexual dimorphism in head size and shape with such methodology are still incipient for anurans (e.g., Vukov *et al.*, 2018a,b). Therefore, proximate causes of allometric scaling in anurans head are still unexplored (Vukov *et al.*, 2018b) and demand further investigation.

Three types of allometry are currently distinguished: (1) ontogenetic allometry – growth is the source of morphological variation, (2) static allometry – reflects covariation of traits among individuals at a particular ontogenetic stage and within a single population, and (3) evolutionary allometry – addresses the variation among phylogenetic lineages considering a similar ontogenetic stage (Cock, 1966; Klingenberg, 1998). The effects of allometric ontogenetic changes on morphology have been examined primarily in larval stages of anurans (e.g., Larson 2002, 2004, 2005); however, the allometric influence (both ontogenetic and static) in post-metamorphic head has been poorly explored so far (e.g., Ponssa and Vera Candioti, 2012; Vukov *et al.*, 2018a,b; Duport-Bru *et al.*, 2019; Sanna, 2019) and the studies which did so are usually focused on interspecific differences (e.g., Ponssa and Vera Candioti, 2012; Duport-Bru *et al.*, 2019; Sanna, 2019).

The genus *Aparasphenodon* Miranda-Ribeiro, 1920 comprises five species (Frost, 2020) with distribution mainly along the Atlantic coast of Brazil (Neto and Teixeira Jr., 2012; Assis *et al.*, 2013). *Aparasphenodon* spp. are known as "casque-headed frogs" due to their heavily ossified skulls bearing cranial crests, ridges and flanges (Trueb, 1970). Such skull features have most likely evolved as adaptations to habitats with scarce water (Trueb, 1970) or associated to phragmotic behaviors (e.g. using the head to plug burrows) (Pimenta *et al.*, 2009). *Aparasphenodon brunoi* Miranda-Ribeiro, 1920 is the most well-known taxon of the genus, with several studies focusing on its ecological and osteological aspects (e.g. Trueb, 1970; Andrade and Abe, 1997; Teixeira *et al.*, 2002; Mesquita *et al.*, 2004; Wogel *et al.*, 2006; Jared *et al.*, 2015; Carmo and Woitovicz-Cardoso, 2018). Sexual dimorphism in overall size and in some external characters (Teixeira *et al.*, 2002; Mesquita *et al.*, 2004), as well as ontogenetic variation on dermal bones (Trueb, 1970), have previously been reported in the literature for the species with the use of traditional morphometric analyses.

Geometric Morphometrics offers precise morphological description and provides effective means for visualization, interpretation and communication of the results (Zelditch *et al.*, 2004). Herein we aim to test two main hypotheses through the use of such technique: (1) There are both ontogenetic and static allometry in the head of *Aparasphenodon brunoi* – this hypothesis is based on the fact that many studied vertebrates present conspicuous allometry in such region (Meyer, 1990; Zeffer *et al.*, 2003; Monteiro *et al.*, 2005; Ponssa and Vera Candioti, 2012; Prevosti *et al.*, 2012; Murta-Fonseca and Fernandes, 2016; Murta-Fonseca *et al.*, 2019); and (2) *A. brunoi* presents sexual dimorphism in head shape – we based this hypothesis considering that females and males tend to differ in size as well as other external characters (Teixeira *et al.*, 2002; Mesquita *et al.*, 2004). We believe that such morphological variation studies are extremely relevant to provide in-depth knowledge about the causes of taxa morphological complexity (Vukov *et al.*, 2018a).

Materials and methods

We analyzed 75 specimens of *A. brunoi* from Parque Nacional da Restinga de Jurubatiba (22°16'15"S; 41°38'49"W), a sandy coastal environmental in the municipalities of Macaé, Carapebus and Quissamã, state of Rio de Janeiro, southeastern Brazil. Specimens examined are deposited in the amphibian collection of Museu Nacional, Universidade Federal do Rio de Janeiro, state of Rio de Janeiro, Brazil (MNRJ). Referred specimens and data on sex and snout-vent length (SVL) are listed in the Appendix I.

Each individual of *A. brunoi* was photographed twice for head dorsal view, by the same person (LFC) with a time interval, in a Leica M205C stereoscope coupled to a DFC 450 camera. Each picture was then duplicated, generating a total of four images of the same individual. *Aparasphenodon brunoi* has an ossified head with cranial formations of crests, ridges, and flanges which can be seen on the dorsal surface of the head without any kind of preparation due its co-ossification (Trueb, 1970). We selected 26 landmarks based on their ability to represent geometric form and being easily recognized and reproduced (Souto *et al*., 2019) – the landmarks were analyzed with object symmetry (Fig. 1; Table 1). We used the software TPSUtil 1.4 (Rohlf, 2008) to compile and convert image files to be analyzed; TPSDig version 2.16 (Rohlf, 2010) for landmarks digitization; and MorphoJ 2.0 (Klingenberg, 2011) to superimpose landmark configurations through generalized Procrustes analysis (Rohlf and Slice, 1990), to generate a covariance matrix, and to perform the GM analyses. A multivariate analysis of variance (MANOVA) was performed in Geomorph package 3.0.7 (Adams *et al*., 2017) of R software (R Core Team, 2014). The osteological terminology follows Trueb (1970). In order to test the error of the position of the specimens during photograph and landmark digitization, we performed an analysis of variance (Procrustes ANOVA) through the comparison of two different photographs of the same specimen and two different landmark digitizations of the same photograph (see Klingenberg and McIntyre, 1998; Klingenberg *et al*., 2002; and Klingenberg, 2015 for details on Procrustes ANOVA).

Five different approaches were made to explore the intraspecific variation in *A. brunoi* head: (1) ontogenetic and (2) static allometries, through a regression analysis of Procrustes coordinates on centroid size. For such purposes, we categorized adults, juveniles, males and females based on Mesquita *et al*. (2004). Categorizations were made through direct observation of gonads, vocal slits, nuptial pads and SVL. For static allometry, we selected 29 adult females and 29 adult males (n=58), whereas for ontogenetic allometry 17 juveniles (9 females and 8 males) were added to this sample, encompassing a total range of 23.5 to 80.2 mm SVL. The third approach – (3) overall shape – was assessed through Principal Component Analysis (PCA) of the Procrustes coordinates; the residual shape (size corrected) (4) was assessed through PCA of the regression residuals; and the sexual dimorphism of adults (5) was assessed through a discriminant analysis and a leave-one-out cross-validation test (Lachenbrunch, 1967). All analyses were made based on the symmetric component. We compared the mean shape of each sex to graphically represent and describe the shape of females and males. Furthermore, we

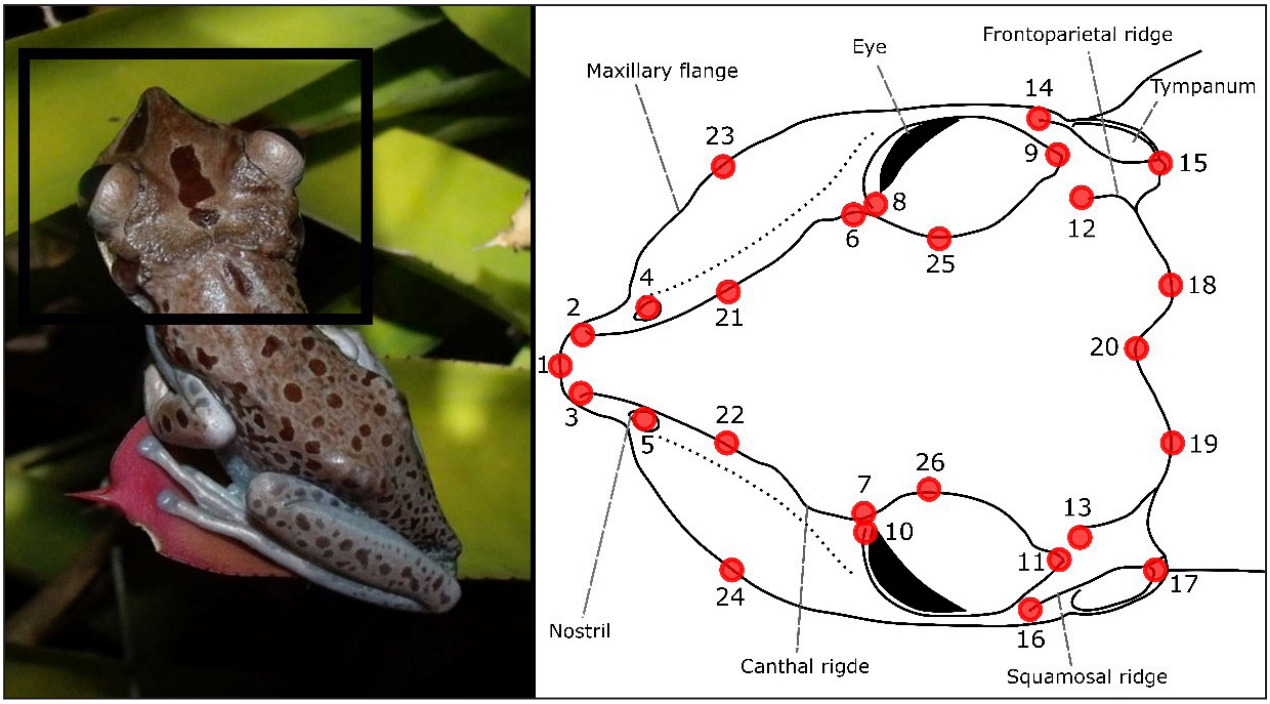

Figure 1. Dorsal view of the head of *A. brunoi* with digitized landmarks for the GM analyses. Photo by Ana Carolina C. Lourenço.

Table 1. Description of landmarks used in the GM analyses of head dorsal view of *Aparasphenodon brunoi*.

Landmark	Description
1	Anteriormost point of snout
2, 3	Anteriormost point of canthal ridge
4, 5	Nostrils
6, 7	Lateralmost point of canthal ridge lateral posterolateral projection
8, 10	Anteriormost point of the eye globe
9, 11	Posteriormost point of the globe eye
12, 13	Posterolateral point of the frontoparietal ridge
14, 16	Anteriormost point of the squamosal ridge
15, 17	Posteriormost point of the squamosal ridge
18, 19	Posterolateral points of the frontoparietal posterior ridge
20	Posterormedial point of the frontoparietal posterior ridge
21, 22	Median points between landmarks 2 and 6 and 3 and 7
23, 24	Maxillary flanges at the level of landmarks 21 and 22
25, 26	Median point of the frontoparietal lateral ridge

performed a MANOVA, considering centroid size, sex, and the interaction between both parameters, in order to evaluate the presence of sexual dimorphism within the species. Considering the different allometric slopes between females and males, the regression analysis was performed within each sex in MorphoJ, through the tool "pooled regression within subgroups". The regression was performed separately for females and males for the description of the allometric trajectories.

Results

The ANOVA analysis resulted in a smaller difference between photograph and landmark-digitization in comparison to the difference between individuals (Table 2). For this reason, the error was considered negligible and the mean Procrustes coordinates of the four images of each individual was used in subsequent analyses. The MANOVA analysis showed a significant result for the interaction between sex and centroid size, which means that the allometric slopes of both sexes are different (Table 3).

For the ontogenetic allometry, the null hypothesis of isometric growth was rejected for both females and males ($p<0.05$). For females, 53.19% of all shape variation was an effect of size, while for males the value was 40.84%. Although the slopes were different for both sexes, the observable shape changes associated to size were the same: larger centroid sizes were related to longer snouts, slightly anteromedially shifted nostrils, larger maxillary flanges, smaller eyes, posterolateral portion of frontoparietal ridge enlarged, and squamosal ridge medially shifted (Fig. 2).

Considering the static allometry, the null hypothesis of isometric growth was also rejected for both sexes ($p<0.05$). For females, 7.37% of adult shape variation was an effect of size increase, while for males the value was 8.5%. Larger centroid sizes showed, in both sexes, posteromedially displaced nostrils, larger maxillary flanges, and wider posterior region of frontoparietal ridges ("helmet") (Fig. 3). Considering our results, females tend to grow larger than males.

The first two principal components were responsible for 36.9% of all shape variation considering overall shape. The first component (22.9% of total variation) showed – in the negative extreme – wide and short heads, with smaller eyes positioned more laterally, wider frontoparietal ridges, and squamosal ridges laterally shifted. The PC2 (14%) shows – in the negative values – longer snout, larger maxillary flanges, canthal ridge process posteriorly shifted, and posterolateral portion of the frontoparietal ridge anteromedially shifted (Fig. 4). Although female and males' components overlap in all axes, PC1 *vs*. PC2 graphic tends to spread both groups, with females exhibiting more negative values in both axes.

Considering the size corrected PCA, the first two components accounted for 35.8% of all shape variation. The PC1 (18.8% - Fig. 5) showed the same variation as in the PC1 of the overall shape analysis (Fig. 4), although less conspicuous. The PC2 (17% - Fig. 5) exhibited about the same modifications as in the PC2 of the overall shape (Fig. 4).

Besides the interaction of sex and centroid size, the MANOVA also showed a significant value for sex solely, meaning that there are differences in the head shape of females and males. In the discriminant analysis of the size corrected shape, permutation test showed a significant Procrustes distance between both sexes ($p<0.05$), with the distance between the mean of each sex of 0.01758657. All females were correctly identified, while only two males were misidentified (Fig. 6a). In the cross-validation test, 87% of the females and 76% of the males were correctly identified (Fig. 6b). In general, females showed

Table 2. Measurement error – Procrustes ANOVA considering the difference of Procrustes coordinates between two photographs of the same individuals and two landmark digitization of the same photograph. Sum of squares (SS), mean squares (MS), degree of freedom (DF), F statistics, and P-value.

Effect	SS	MS	DF	F	P (param.)
Individual	0.55531686	0.0003085094	1800	7.18	<.0001
Side	0.02852880	0.0011887000	24	27.67	<.0001
Ind*Side	0.07731553	0.0000429531	1800	1.38	<.0001
Photograph	0.10034666	0.0000312023	3216	1.48	<.0001
Landmark	0.13998549	0.0000211331	6624		

slightly anteriorly positioned nostrils, smaller eyes, larger canthal ridges, narrower and longer frontoparietal ridges, and larger and laterally shifted squamosal ridges (Fig. 6c, d). Males show the opposite features (Fig. 6e, f). The distance between the mean of each sex was larger than the average distance between two random adult individuals of the same sex (0.0003078318 for females and 0.0002940567 for males).

Discussion

This study evaluates - through the use of GM - the allometry, sexual dimorphism, and morphological variation of *Aparasphenodon brunoi*. We found significant allometry in the head shape of the species (both ontogenetic and static), with 40-53% of all variation related to changes in size through development in both sexes. We also found that the allometric slopes vary in females and males. This result could indicate that some sort of selection, as sexual selection, is causing the differences in the allometric slopes between sexes, which is, in turn, contributing to a sexual dimorphism – a relation that was already found in other organisms (Voje and Hansen, 2012). According to our results, three different sexual dimorphic traits are present in *A. brunoi* head: size, shape, and allometric slopes.

Allometry

Zeng (1988) provided a model to explain the evolution of allometry, indicating that shifts in directional selection on body size in one sex and correlated shifts in selection on body size in the other sex can lead to the evolution of interspecific allometry. Correlation between selection on male body size and selection on female body size may arise, for example, through shared ecology or the mechanics of reproduction (Lisle and Rowe, 2013). Key features of this model are its prediction that the sex under more intense direct selection will be the more phenotypically divergent and that the response in the second sex will be weaker (Lisle and Rowe, 2013). Thus, the model predicts female-divergent allometry where there is stronger direct selection on female size (Fairbairn, 1997; Lisle and Rowe, 2013). In this sense, female biased sexual size dimorphism, as seen here, may evolve primarily through negative directional selection on male body size or positive directional selection on female body size (Lisle and Rowe, 2013). In a study that investigated such features for more than 1,000 amphibian species, Lisle and Rowe (2013) found that the sexual size dimorphism in this taxon is generally female biased, and a correlation between allometric slopes and sexual size dimorphism supports the hypothesis that selection on female body size has played a key role in generating such variation in amphibians.

Our sample shows females with larger heads than males, as found in their SVL and in previous studies of anurans (e.g., Rivas and Burghardt, 2001; Mesquita *et al*., 2004), corroborating Lisle and Rowe (2013) data. Our data also supports the idea that the sexual and natural selection for larger females

Table 3. MANOVA considering centroid size, sex, and the interaction between these parameters for *Aparasphenodon brunoi* head shape. Sum of squares (SS), mean squares (MS), degrees of freedom (DF), R squared coefficient (RSQ), F statistics, effect-size (Z), and P-value.

	DF	SS	MS	RSQ	F	Z	Pr(>SS)
Centroid	1	0.070366	0.070366	0.36665	45.3298	6.1161	0.001
Sex	1	0.006304	0.006304	0.03285	4.0609	4.0726	0.001
Centroid*Sex	1	0.003481	0.003481	0.01814	2.2423	2.4209	0.006
Residuals	72	0.111767	0.001552	0.58237			
Total	75	0.191917					

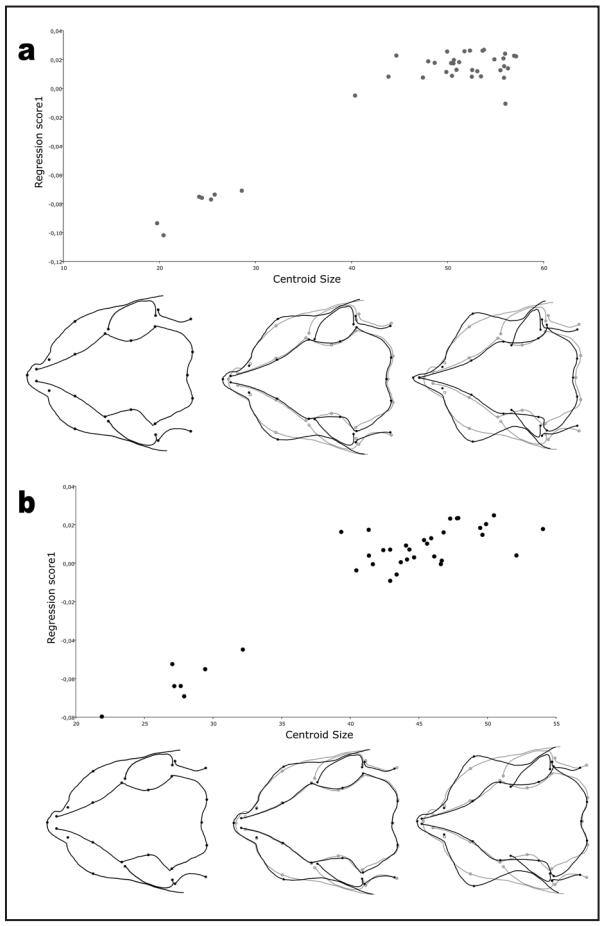

Figure 2. Ontogenetic allometry (whole sample) assessed through regression analysis of the Procrustes coordinates of the dorsal view of the head of *A. brunoi* females (a) and males (b). Grey dots females; black dots males. Shapes on the bottom represent the smaller to larger centroid size, respectively – black lines represent the target and grey lines the mean shape. Asymmetries on the soft outline are merely an artifact of the graphic output.

– which could, for example, harbour more eggs – changed the allometric slopes between females and males and corroborated the sexual dimorphism observed. Different allometric slopes as the source of sexual dimorphism is also known in other species of anurans, as the Bufonidae *Rhinella rubescens* and *R. diptycha* (Arantes *et al.*, 2015). On the other hand, Vukov et al (2018a), investigating sexual dimorphism in the skull of the yellow-bellied toad (*Bombina variegata*), found no differences in the allometric slopes of females and males, but about the same amount of sexual dimorphism found herein (see further in the discussion).

Ontogenetic head/skull shape variation based on GM has been explored in several ways in anuran larval stages (e.g., Larson, 2002, 2004, 2005; Garriga and Llorente, 2012) and post-metamorphic individuals (e.g., Birch, 1999; Ponssa and Vera Candioti, 2012; Vukov *et al.*, 2018b). Such studies, however, were conducted so far to the families Leptodactylidae (Ponssa and Vera Candioti, 2012; Duport-Bru *et al.*, 2019) and Bufonidae (Birch, 1999; Sanna, 2019) and no studies of this kind have ever been performed for hylid frogs. As in our results, such available reports have also recovered a reduction of eye size during post-metamorphic development (Vukov *et al.*, 2018b), which is expected for vertebrates as a result of the reduction of relative size of sensory structures coupled with proportional enlargement of the facial/rostral region (Emmerson and Bramble, 1993; Kaliontzopoulou *et al.*, 2007). Such changes during development of the head have also been found in other areas associated to sensory organs in anurans, as the posterior region of the head (region of squamosal, associated to the otic region) and snout (olfactory organs) (e.g., our study; Vukov *et al.*, 2018b, Duport-Bru *et al.*, 2019). The allometric changes found herein follow a common pattern of vertebrates, with a modular arrangement

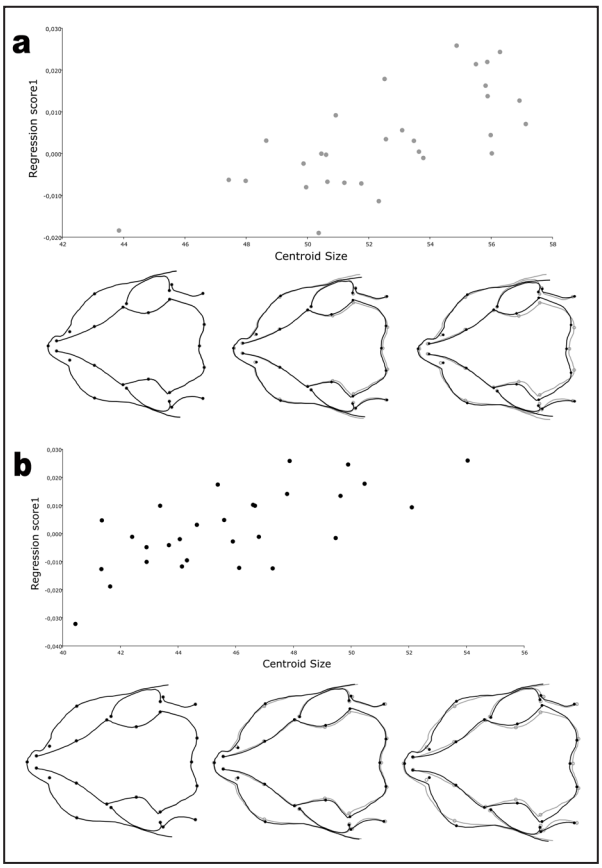

Figure 3. Static allometry (only adults) assessed through regression analysis of the Procrustes coordinates of the dorsal view of the head of *A. brunoi* females (a) and males (b). Grey dots females; black dots males. Shapes on the bottom represent the smaller to larger centroid size, respectively – black lines represent the target and grey lines the mean shape. Asymmetries on the soft outline are merely an artifact of the graphic output.

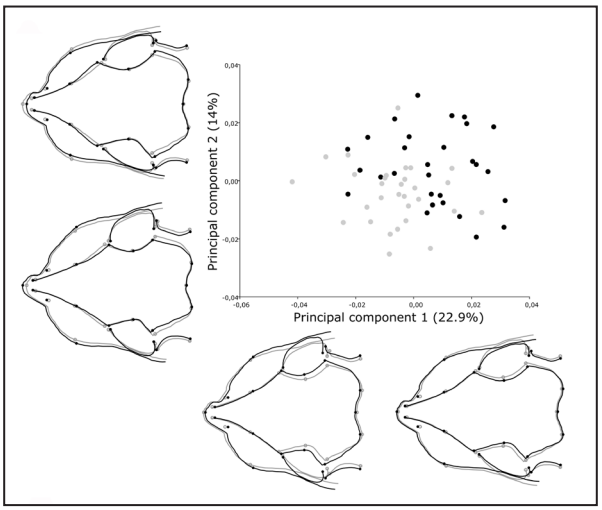

Figure 4. Principal component analysis considering overall shape in *A. brunoi* (only adults). Shapes represent, respectively in counterclockwise, PC2 in 0.04, PC2 in -0.06, PC1 in -0.06 and PC1 in 0.04 – black lines represent the target and grey lines the mean shape. Grey dots females; black dots males. Asymmetries on the soft outline are merely an artifact of the graphic output.

where the neurocranium becomes smaller, while the snout tends to be proportionally larger with growth, besides an anteromedially displacement of nostrils (Emerson and Bramble, 1993; Hanken and Hall, 1993; Duport-Bru *et al*., 2019). The amount of shape changes associated to size during growth found herein is also consistent with most of the previous studies regarding this matter (e.g., Birch, 1999; Ponssa and Vera Candioti, 2012; Vukov *et al*., 2018b; Sanna, 2019). Such patterns are expected considering that different anatomical regions grow at different rates in order to maintain function that could possibly be lost in the case of isometric growth (Emerson and Bramble, 1993; Vukov *et al*., 2018b).

Our results show an influence of static allometry in shape diversity around 7-8% for both males and females, which is very similar to the results obtained from Vukov *et al*. (2018a) and to other studies of vertebrates (e.g., Murta-Fonseca *et al*., 2019). Understanding such allometric changes is crucial for anurans since this group goes through profound morphological changes during metamorphosis, and their skull is structurally repatterned from a larval skull adapted to an aquatic life to a skull adapted to terrestrial life, with functional shifts in feeding, breathing and sensorial system (Hanken and Summers, 1988; Duellman and Trueb, 1994; Vukov *et al*., 2018a;). Therefore, early and late cranial growth periods probably are not correlated, and shapes could be stage specific (Vukov *et al*., 2018a).

Observed modifications throughout ontogenetic series might be related to variations in dermal bones, as previously reported for the species (Trueb, 1970). Juveniles tend to have external nostrils near the end of the snout probably due to the late development of prenasal, which is fully developed only in the adult [head length 25.4 mm, according to Trueb (1970)], when prenasal extends anteriorly beyond external nostrils, making the snout highly acuminate. Trueb (1970) found visible parotic crests in dorsal view of small individuals, a feature that is an outcome of the posterolateral portion of the frontoparietal ridge poorly developed; the anterior and posterior arms of squamosal presented poorly developed dorsal flanges, with the growth of frontoparietal and squamosal flanges articulating with each other in the larger individuals. In such larger specimens (Trueb, 1970) there was an extensive posterolateral development of the frontoparietals. In the juvenile specimens, the maxillaries were widely separated anteriorly, while in a fully developed adult the maxillaries were narrowly separated medially (Trueb, 1970). Except for the last ontogenetic changes mentioned (medial separation of maxillaries), all aforementioned ontogenetic variations were also found as significant ontogenetic shifts in the present study, for both females and males, reflecting the ontogenetic development of the bones.

Sexual dimorphism

Most of the current available studies regarding post-metamorphic amphibian sexual dimorphism in

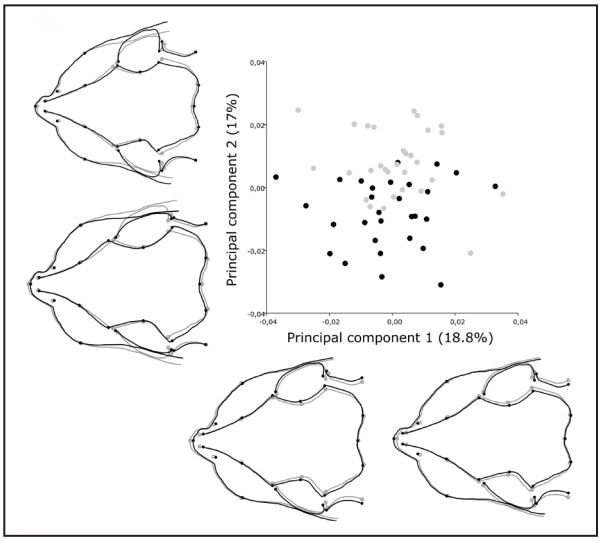

Figure 5. Size corrected principal component analysis of *A. brunoi* (only adults). Shapes represent, respectively in counterclockwise, PC2 in 0.04, PC2 in -0.04, PC1 in -0.04 and PC1 in 0.04 – black lines represent the target and grey lines the mean shape. Grey dots females; black dots males. Asymmetries on the soft outline are merely an artifact of the graphic output.

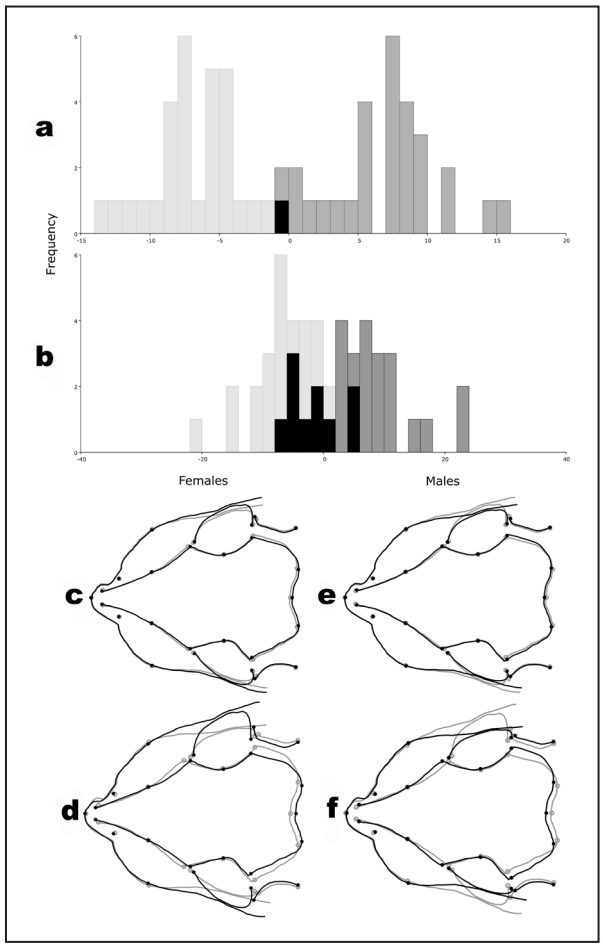

Figure 6. Discriminant analysis (a) and cross-validation (b) test with size corrected head shape of the head of *A. brunoi*. X-axis represents the vector of Fisher's discriminant rule, with the cut-off point at a value of zero – females with positive values were found within male configurations in the cross-validation test, while males with negative values were found within female configurations. Light grey females; dark grey males; black females and males. Mean shape females (c and d) and males (e and f) – c and e represent the real mean and d and f the difference enlarged by 3 times. Asymmetries on the soft outline are merely an artifact of the graphic output.

head/skull size and/or shape is based on linear morphometry (e.g., Katsikaros and Shine, 1993; Teodecki *et al.*, 1998; Kupfer, 2009; Ponssa and Medina, 2016). Little has been explored in that sense through the use of GM, with some studies focusing on salamanders and newts (e.g., Ivanovic and Kalezic, 2012; Alcorn *et al.*, 2013). Data on anurans are still scarce and recent and, to the best of our knowledge, are based exclusively on Vukov *et al.* (2018a).

The amount of divergence between females and males shape found herein was very similar to that found by Vukov *et al.* (2018a) [around 0.017 here and 0.016 in Vukov *et al.* (2018a), in Procrustes distance]. The similarities of the only two studies of cranial/head sexual dimorphism using GM in anurans (Vukov *et al.*, 2018a and this study) point to the importance of further investigations assessing if such results represent a pattern for anurans. Vukov *et al.* (2018a), however, found males with slightly larger craniums than females, in opposition to what was found in the present study. In the case of the yellow-bellied toad (Vukov *et al.*, 2018a), breeding males are usually involved in territorial behavior, such as short-term spawning aggregations, what can be one of the explanations to the larger heads of the sex. This kind of behavior is not known for *A. brunoi* and, in this case, the pattern of a larger head in females could be only following an also larger body, which appears to be a common trait in amphibians (e.g., Kupfer, 2009).

After correcting our data for the size, the non-allometric shape divergence in the head of *A. brunoi* points out to shape dimorphism mostly associated to the maxilla (maxillary flanges) and posterior skull elements (frontoparietal). Although maxillary shape divergences are traditionally associated to trophic demands in anurans (e.g., Vukov *et al.*, 2018a), the absence of previously reported sexual driven divergences for the species (Mesquita *et al.*, 2004) suggests different dimorphic demands, most likely not associated to feeding. Broader maxillary flanges might also contribute to a more effective bromeliad sealing for females, minimizing evaporative water loss (Andrade and Abe, 1997) as a presumably higher demand on egg development and/or spawning. According to Mesquita *et al.* (2004), there is also an evident correlation between anuran head measurements (=size) and bromeliads size. Considered as one of the largest anurans inhabiting bromeliads in the sandy coastal plains, *A. brunoi* exhibits a phragmotic behavior, in which the body is kept protected in the bromeliad tank while the skull serves as a 'cap'. Our results thus emphasize that variation of specific morphological traits might play an important role in the choice and occupation of bromeliads by females and males, maybe minimizing the competition to shelter between sexes, what could be investigated in further studies. Both the capacity of minimizing water loss and the minimization of competition to shelter between females and males could influence the fitness of the species and select such morphological aspects through the evolutionary history of *A. brunoi*. The influence conditions of the physical environment as a source of morphological variation has already been assessed through GM for anuran populations (e.g., Bandeira *et al.*, 2016), and thus

experimental approaches are needed to assess such responses to the habitat conditions.

The intraspecific morphological variation found in *A. brunoi* (including allometry and sexual dimorphism) highlights the importance of studies assessing such data. The lack of data about cranial sexual size and shape dimorphism keeps us far from large comparative studies and an ultimate ecological understanding of sex-specific cranium evolution (Vukov *et al*., 2018a). These studies are important on providing valuable information on the species systematics, as well as on understanding factors that lead to polymorphic characters, which are genetically driven or even environmentally influenced and therefore, provide fundamental knowledge on anuran species ecology and evolution.

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Appendix 1

Material Examined
Aparasphenodon brunoi (SVL-mm)
Females: MNRJ 92559 (75.0); 92599 (75.6); 92600 (74.7); 92602 (76.6); 92614 (66.7); 92617 (76.7); 92618 (73.4); 92621 (73.4); 92623 (69.0); 92625 (62.6); 92627 (68.6); 92629 (67.4); 92630 (80.2).

Males: MNRJ88018 (57.8); 88020 (56.8); 88021 (61.0); 88026 (62.0); 89268 (58.8); 92548 (55.7); 92549 (61.5); 92551 (65.7); 92552 (59.0); 92553 (57.9); 92554 (54.0); 92555 (62.8); 92564 (68.3); 92598 (55.3); 92605 (56.0); 92608 (47.4); 92611 (55.0); 92612 (51.1); 92622 (55.4); 92624 (50.3); 92626 (62.9); 92628 (67.4).